

CLAIMS

WE CLAIM:

1. A method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell at least one double-stranded RNA effector molecule comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T.
2. The method of claim 1, wherein at least two double-stranded RNA effector molecules are administered to said cell, each comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO: 49.
3. The method of claim 2, comprising administering to said cell double-stranded RNA effector molecules comprising SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO: 49.
4. The method of claim 1, wherein said administering is accomplished by providing to the *in vivo* mammalian cell at least one expression vector capable of expressing at least one double-stranded RNA effector molecule comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:49.
5. The method of claim 4, comprising providing to the *in vivo* mammalian cell at least one expression vector capable of expressing at least two double-stranded RNA effector molecules comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO: 49.

6. The method of claim 5, comprising expressing at least four double-stranded RNA effector molecules comprising a sequence selected from the group consisting of SEQ NO:18, SEQ ID NO:19, SEQ ID NO:23, and SEQ ID NO: 49.
- 5 7. The method of claim 4, wherein said at least one expression vector comprises a promoter selected from the group consisting of an RNA polymerase I promoter, an RNA polymerase II promoter, a T7 polymerase promoter, an SP6 polymerase promoter, an RNA polymerase III promoter, a tRNA promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding one or more of said double-stranded RNA effector molecules.
- 10 8. The method of claim 1, wherein the mammalian cell is a human cell.
- 15 9. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising at least one double-stranded RNA effector molecule, each double-stranded RNA effector molecule comprising a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:49; wherein U is substituted for T.
- 20 10. A composition of claim 9, comprising at least one expression vector capable of expressing at least one of said double-stranded RNA effector molecules in a mammalian cell.
- 25 11. A composition of claim 10, wherein the expression vector comprises at least one promoter selected from the group consisting of a polymerase I promoter, a polymerase III promoter, a U6 promoter, an H1 promoter, a 7SK promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding one or more of said double-stranded RNA effector molecules.

12. A composition of 9, comprising at least one expression vector capable of expressing in an *in vivo* mammalian cell a double-stranded RNA effector molecule comprising SEQ ID NO:18; a double-stranded RNA effector molecule comprising SEQ ID NO:19; a double-stranded RNA effector molecule comprising SEQ ID NO:23; and a double-stranded RNA effector molecule comprising SEQ ID NO:49; wherein U is substituted for T.
- 5 13. A mammalian cell comprising an expression vector of claim 10.
- 10 14. A method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell at least two double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising: (a) a sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:62; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T.
- 15 15. The method of claim 14, wherein said at least two double-stranded RNA effector molecules are administered to the cell by providing at least one expression vector encoding the double-stranded RNA effector molecules.
- 20 16. The method of claim 15, wherein the double-stranded RNA effector molecules are hairpin dsRNA molecules.
- 25 17. The method of claim 15, wherein the expression vector comprises at least one promoter selected from the group consisting of a polymerase I promoter, a polymerase III promoter, a U6 promoter, an H1 promoter, a 7SK promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding one or more of said double-stranded RNA effector molecules.

18. A composition for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising at least two double-stranded RNA effector molecules, each double-stranded RNA effector molecule comprising: (a) a sequence selected from the group consisting of SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, and SEQ ID NO:62; (b) the reverse complement of said selected sequence; and (c) optionally, a sequence linking sequences (a) and (b); wherein U is substituted for T.

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19. The composition of claim 18, comprising at least one expression vector encoding said at least two double-stranded RNA effector molecules.

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20. The composition of claim 19, wherein the double-stranded RNA effector molecules are hairpin dsRNA molecules.

21. The composition of claim 19, wherein the expression vector comprises at least one promoter selected from the group consisting of a polymerase I promoter, a polymerase III promoter, a U6 promoter, an H1 promoter, a 7SK promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding one or more of said double-stranded RNA effector molecules.

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22. A method for inhibiting expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising administering to said cell at least two double-stranded RNA effector molecules comprising: (a) an RNA sequence equivalent to a hepatitis C virus DNA coding strand sequence selected from the group consisting of sequence position 9510-9531, 9510-9533, 9510-9534, 9510-9535, 9510-9536, 9514-9534, 9514-9535, 9514-9536, 9514-9539, 9514-9540, 9514-9542, 9517-9539, 9517-9540, 9517-9542, 9517-9544, 9518-9539, 9518-9540, 9518-9542, 9518-9544, 9520-9540, 9520-9542, 9520-9544, 9520-9548, 9521-9542, 9521-9544, 9521-9548, 9521-9549, 9522-9542, 9522-9544, 9522-9548, 9522-

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9549, 9527-9548, 9527-9549, 9527-9551, 9527-9552, 9527-9553, 9527-
9555, 9528-9548, 9528-9549, 9528-9551, 9528-9552, 9528-9553, 9528-
9555, 9530-9551, 9530-9552, 9530-9553, 9530-9555, 9530-9557, 9530-
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5 9559, 9532-9560, 9537-9557, 9537-9558, 9537-9559, 9537-9560, 9537-
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9569, 9543-9564, 9543-9566, 9543-9568, 9543-9569, 9543-9571, 9545-
9566, 9545-9568, 9545-9569, 9545-9571, 9545-9573, 9546-9564, 9546-
10 9566, 9546-9569, 9546-9571, 9546-9573, 9547-9568, 9547-9569, 9547-
9571, 9547-9573, 9547-9575, 9550-9571, 9550-9573, 9550-9575, 9550-
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15 9588, 9563-9589, 9563-9591, 9565-9586, 9565-9587, 9565-9588, 9565-
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9591, 9567-9593, 9567-9595, 9570-9591, 9570-9593, 9570-9595, 9570-
9596, 9570-9598, 9572-9593, 9572-9595, 9572-9596, 9572-9598, 9574-
9595, 9574-9596, 9574-9598, 9574-9601, 9576-9596, 9576-9598, 9576-
20 9601, 9576-9604, 9579-9601, 9579-9604, 9581-9601, 9581-9604, and
9583-9604; (b) an RNA sequence which is the reverse complement of the
selected hepatitis C virus DNA coding strand sequence; and, optionally,
(c) a sequence linking (a) and (b).

23. The method of claim 22, wherein said administering is accomplished
25 by providing one or more expression vectors capable of expressing in said
mammalian cell said at least two double-stranded RNA effector molecules.

24. The method of claim 23, wherein said one or more expression vectors
comprise at least one promoter selected from an RNA polymerase I
promoter, an RNA polymerase II promoter, a T7 polymerase promoter, an
30 SP6 polymerase promoter, an RNA polymerase III promoter, a tRNA

promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding at least one of said double-stranded RNA effector molecules.

25. The method of Claim 24, wherein at least one expression vector
5 comprises at least two expression cassettes, each expression cassette comprising at least one RNA polymerase III promoter selected from the group consisting of a U6 promoter, a 7SK promoter, an H1 promoter, and an MRP promoter.

26. The method of claim 22, wherein the mammalian cell is a human cell.

10 27. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising at least two double-stranded RNA effector molecules, each comprising: (a) an RNA sequence equivalent to a hepatitis C virus DNA coding strand sequence selected from the group consisting of sequence position 9510-
15 9531, 9510-9533, 9510-9534, 9510-9535, 9510-9536, 9514-9534, 9514-9535, 9514-9536, 9514-9539, 9514-9540, 9514-9542, 9517-9539, 9517-9540, 9517-9542, 9517-9544, 9518-9539, 9518-9540, 9518-9542, 9518-9544, 9520-9540, 9520-9542, 9520-9544, 9520-9548, 9521-9542, 9521-9544, 9521-9548, 9521-9549, 9522-9542, 9522-9544, 9522-9548, 9522-
20 9549, 9527-9548, 9527-9549, 9527-9551, 9527-9552, 9527-9553, 9527-9555, 9528-9548, 9528-9549, 9528-9551, 9528-9552, 9528-9553, 9528-9555, 9530-9551, 9530-9552, 9530-9553, 9530-9555, 9530-9557, 9530-9558, 9532-9552, 9532-9553, 9532-9555, 9532-9557, 9532-9558, 9532-9559, 9532-9560, 9537-9557, 9537-9558, 9537-9559, 9537-9560, 9537-
25 9561, 9537-9564, 9538-9558, 9538-9559, 9538-9560, 9538-9561, 9538-9564, 9538-9566, 9541-9561, 9541-9564, 9541-9566, 9541-9568, 9541-9569, 9543-9564, 9543-9566, 9543-9568, 9543-9569, 9543-9571, 9545-9566, 9545-9568, 9545-9569, 9545-9571, 9545-9573, 9546-9564, 9546-9566, 9546-9569, 9546-9571, 9546-9573, 9547-9568, 9547-9569, 9547-

9571, 9547-9573, 9547-9575, 9550-9571, 9550-9573, 9550-9575, 9550-
9577, 9550-9578, 9554-9575, 9554-9577, 9554-9578, 9554-9580, 9556-
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9596, 9570-9598, 9572-9593, 9572-9595, 9572-9596, 9572-9598, 9574-
9595, 9574-9596, 9574-9598, 9574-9601, 9576-9596, 9576-9598, 9576-
10 9601, 9576-9604, 9579-9601, 9579-9604, 9581-9601, 9581-9604, and
9583-9604; (b) an RNA sequence which is the reverse complement of the
selected hepatitis C virus DNA coding strand sequence; and, optionally,
(c) a sequence linking (a) and (b).

28. An expression vector encoding a composition of claim 27.

15 29. A mammalian cell comprising an expression construct of claim 28.

30. An expression vector of claim 28 comprising at least one promoter
selected from an RNA polymerase I promoter, an RNA polymerase II
promoter, a T7 polymerase promoter, an SP6 polymerase promoter, an
RNA polymerase III promoter, a tRNA promoter, and a mitochondrial
20 promoter, said promoter operably linked to a sequence encoding at least
one of said double-stranded RNA effector molecules.

31. An expression vector of claim 30 comprising at least two expression
cassettes, each expression cassette comprising at least one RNA
polymerase III promoter selected from the group consisting of a U6
25 promoter, a 7SK promoter, an H1 promoter, and a MRP promoter, each of
said RNA polymerase III promoters operably linked to a sequence
encoding a double-stranded RNA effector molecule.

32. A method for inhibiting expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising administering to said cell a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence
5 selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T.

33. The method of claim 32, wherein at least two of said double-stranded
10 RNA effector molecules are administered to the same mammalian cell.

34. The method of claim 33, wherein said at least two double-stranded RNA effector molecules comprise an at least 19 contiguous base pair
15 nucleotide sequence from within more than one of SEQ ID NO:1 through SEQ ID NO:10.

35. The method of claim 34, wherein said administering is accomplished by providing one or more expression vectors capable of expressing in said
20 mammalian cell said at least two double-stranded RNA effector molecules.

36. The method of claim 35, wherein said one or more expression vectors further comprise a promoter selected from an RNA polymerase I promoter, an RNA polymerase II promoter, a T7 polymerase promoter, an SP6
25 polymerase promoter, an RNA polymerase III promoter, a tRNA promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding at least one of said double-stranded RNA effector molecules.

30 37. A method for inhibiting expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising administering to said cell a double-stranded RNA effector molecule comprising an at least

19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:12; wherein U is substituted for T.

- 5 38. The method of claim 37, wherein at least two of said double-stranded RNA effector molecules are administered to the same mammalian cell.
- 10 39. The method of claim 38, wherein said at least two double-stranded effector molecules comprise an at least 19 contiguous base pair nucleotide sequence from within more than one of SEQ ID NO:11; SEQ ID NO:12; and SEQ ID NO: 27.
- 15 40. The method of claim 39, wherein said administering is accomplished by providing one or more expression vectors capable of expressing in said mammalian cell said at least two double-stranded RNA effector molecules.
- 20 41. The method of claim 40, wherein said one or more expression vectors comprise one or more promoters selected from an RNA polymerase I promoter, an RNA polymerase II promoter, a T7 polymerase promoter, an SP6 polymerase promoter, an RNA polymerase III promoter, a tRNA promoter, and a mitochondrial promoter, said promoter operably linked to a sequence encoding at least one of said double-stranded RNA effector molecules.
- 25 42. The method of claim 41, wherein at least one expression vector comprises at least two expression cassettes, each expression cassette comprising at least one RNA polymerase III promoter selected from the group consisting of a U6 promoter, a 7SK promoter, an H1 promoter, and a MRP promoter, each of said RNA polymerase III promoters operably linked to a sequence encoding a said double-stranded RNA effector molecule.

43. A method for inhibiting expression of both a polynucleotide sequence of hepatitis B virus and a polynucleotide sequence of hepatitis C virus in the same *in vivo* mammalian cell, comprising administering to said cell a

5 double-stranded RNA effector molecule comprising a first at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T;

10 and a double-stranded RNA effector molecule comprising a second at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:11; SEQ ID NO:12; and SEQ ID NO: 27; wherein U is substituted for T.

15 44. The method of claim 43, wherein at least two double-stranded RNA effector molecules comprising an at least 19 contiguous base pair nucleotide sequence from within SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; and at least two double-stranded

20 RNA effector molecules comprising an at least 19 contiguous base pair nucleotide sequence from within SEQ ID NO: 11, SEQ ID NO:12, and SEQ ID NO: 27, are administered to the same *in vivo* mammalian cell.

25 45. The method of claim 43, wherein said administering is accomplished by providing one or more expression vectors capable expressing said double-stranded RNA effector molecules in said mammalian cell.

30 46. The method of claim 45, wherein said one or more expression vectors comprise one or more promoters selected from the group consisting of an RNA polymerase I promoter, an RNA polymerase II promoter, a T7 polymerase promoter, an SP6 polymerase promoter, an RNA polymerase III promoter, a tRNA promoter, and a mitochondrial promoter, said

promoter(s) operably linked to a sequence encoding at least one of said double-stranded RNA effector molecules.

47. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis B virus in an *in vivo* mammalian cell comprising a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; wherein U is substituted for T.
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48. The composition of claim 47 comprising at least two double-stranded RNA effector molecules wherein said effector molecules comprise an at least 19 contiguous base pair nucleotide sequence from within more than one of SEQ ID NO:1 through SEQ ID NO:10.
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49. A composition of claim 48, comprising at least two double-stranded RNA effector molecules comprising an at least 19 contiguous base pair nucleotide sequence from within more than one of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
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50. A composition of claim 49, comprising at least three double-stranded RNA effector molecules each comprising an at least 19 contiguous base pair nucleotide sequence from within at least three of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.
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51. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in an *in vivo* mammalian cell comprising a double-stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence
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selected from the group consisting of SEQ ID NO:11 and SEQ ID NO:12; wherein U is substituted for T.

52. The composition of claim 51 comprising at least two double-stranded
5 RNA effector molecules, wherein effector molecules comprising an at least
19 contiguous base pair nucleotide sequence from within more than one of
SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO: 27 are present in the
composition.

10 53. The composition of claim 52 comprising at least one expression
construct capable of expressing the at least two double stranded RNA
effector molecules in an *in vivo* mammalian cell.

15 54. A composition for inhibiting the expression of both a polynucleotide
sequence of hepatitis B virus and a polynucleotide sequence of hepatitis C
virus in a single *in vivo* mammalian cell comprising a double-stranded RNA
effector molecule comprising a first at least 19 contiguous base pair
nucleotide sequence from within a sequence selected from the group
consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4,
20 SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9,
and SEQ ID NO:10; wherein U is substituted for T; and a double-stranded
RNA effector molecule comprising a second at least 19 contiguous base
pair nucleotide sequence from within a sequence selected from the group
consisting of SEQ ID NO:11, SEQ ID NO:12, and SEQ ID NO:27; wherein
25 U is substituted for T.

30 55. The composition of claim 54 comprising at least one expression
construct capable of expressing the at least two double stranded RNA
effector molecules in an *in vivo* mammalian cell.

56. The composition of claim 54 comprising at least two double-stranded
RNA effector molecules comprising an at least 19 contiguous base pair

nucleotide sequence from within SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10; and at least two double-stranded RNA effector molecules comprising an at least 19 contiguous base pair

5 nucleotide sequence from within SEQ ID NO: 11, SEQ ID NO:12, and SEQ ID NO: 27.

57. The composition of claim 56 comprising at least one expression vector capable of expressing said double-stranded RNA effector molecules.

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58. The composition of claim 56 comprising at least two double-stranded RNA effector molecules comprising an at least 19 contiguous base pair nucleotide sequence from within SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO:8.

15 59. A composition of claim 58 comprising at least one expression vector capable of expressing said at least two double-stranded RNA effector molecules in an *in vivo* mammalian cell.

20 60. A polynucleotide sequence comprising a sequence selected from SEQ ID NO:14 through SEQ ID NO:26, and SEQ ID NO:49.

61. A polynucleotide sequence comprising nucleotides 1-19, 1-20, 1-21, 2-20, 2-21, or 3-21 of a sequence selected from SEQ ID NO:14 through SEQ ID NO:26, and SEQ ID NO:49.

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62. A polynucleotide sequence comprising an at least 19 contiguous base pair nucleotide sequence from within a sequence selected from SEQ ID NO:27 through SEQ ID NO:44, SEQ ID NO: 50 through SEQ ID NO:62, and SEQ ID NO: 72 through 76.

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63. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in a mammalian cell, comprising a double-

stranded RNA effector molecule comprising an at least 19 contiguous base pair nucleotide sequence from within SEQ ID NO:27; wherein U is substituted for T.

- 5 64. A composition for inhibiting the expression of a polynucleotide sequence of hepatitis C virus in a mammalian cell, comprising at least one double-stranded RNA effector molecule comprising (a) a sequence selected from the group consisting of SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76, and (b) the reverse complement of said selected sequence; and, optionally, (c) a sequence linking sequences (a) and (b); wherein U is substituted for T.
- 10 65. A composition of claim 64 wherein said at least one double-stranded RNA effector molecule comprises a sequence selected from the group consisting of SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, and SEQ ID NO: 76.
- 15 66. An expression vector encoding a double-stranded RNA effector molecule of claim 64.
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